Effects of the herbicide prometryn on the physicochemical properties of artificial model membranes

A Bertoluzza, S Bonora, G Fini* and M A Morelli

Centro di Studio Interfacoltà sulla Spettroscopia Raman, Department of Biochemistry, University of Bologna, via Belmeloro 8/2, I-40126 Bologna, Italy

Abstract: Several derivatives of 1,3,5-triazine (eg atrazine, prometryn) are effective herbicides and have been extensively used in agriculture. A study of prometryn (Prom)-phospholipid (PL), with different charges, was carried out by means of calorimetric and spectroscopic techniques. The Prom-dipalmitoylphosphatidylcholine (DPPC) and Prom-dipalmitoylphosphatidic acid (DPPA) water suspensions systems at pH values ranging from 5 to 9 were investigated. The results show that Prom does not significantly perturb the hydrophobic core of the lipid bilayer and suggest that the herbicide localizes near the glycerol backbone of the lipid, perturbing the environment of the carbonyls of the two ester groups. At pH 5 the vibrational band attributable to N–H stretch of Prom disappears thus suggesting that the absorption into the bilayer modifies the physicochemical properties of the herbicide. The two PL considered behave similarly and the effect of the charge is not noticeable. The strength of the Prom-PL interaction decreases with decreasing pH.

Keywords: phospholipid; phase transition; herbicides; triazine; prometryn

1 INTRODUCTION

Several derivatives of 1,3,5-triazine are effective herbicides and are extensively used to control weeds selectively in crops, nurseries and orchards, and for non-selective weed control on industrial sites, rights-on-way and irrigation ditches. The *S*-triazine compounds exert their herbicidal properties by interfering with the photosynthesis process in plants; however the details of their mode of action are not fully understood.[1] They seem to inhibit basal electron transport, methylamine-uncoupled electron transport and noncyclic electron transport, with water as the electron donor and ferricyanide or $NADP^+$ as the electron acceptor. These herbicides have also been supposed to inhibit photosynthetic electron transport between the primary PS II electron-accepting quinone and the two-electron gate, a plastoquinone termed B.[2] Studies have indicated that the molecular basis for the resistance of some plants and algae to the triazine derivatives could be due to a change in the DNA coding for one of the proteins found in the PS II complex.[3–5] All these findings have prompted us to suppose that the interaction between triazine derivatives and membrane lipids could play an important role in the herbicidal effects, mainly by modifying the structure of the lipoprotein complexes.

Prometryn (N^2, N^4-di-isopropyl-6-methylthio-1,3,5-triazine-2,4-diamine), a member of the triazine family, is a widely used herbicide and is similar to atrazine in biological properties. However, unlike atrazine, prometryn should not give the hydroxy (bio-inactive) form as a consequence of the protonation of amino groups; indeed the surface amino group of atrazine is protonated when exposed to atmospheric moisture;[6] the protonation of the amino group destabilizes the chloro-atrazine form because the electron density in the vicinity of the protonated amino-group decreases. This renders the C—Cl bond less stable, and Cl can be easily substituted by OH, thus forming protonated hydroxy-atrazine. This fact could modify the mechanism of prometryn action compared to that of atrazine.

Our previous studies on the interaction between bipyridilium derivatives (paraquat, diquat) and phospholipids supported the idea that herbicides can have biomembranes as the primary target for their action;[7] in particular, paraquat, a herbicide positively charged at pH 7, is involved in an electrostatic interaction with lipids, and the interaction is particularly intensive with negatively charged phospholipids (eg DPPA).

In this paper we report studies on the interaction of prometryn (Prom; Fig 1) with dipalmitoylphos-

* Correspondence to: G Fini, Centro di Studio Interfacoltà sulla Spettroscopia Raman, Department of Biochemistry, Univ of Bologna, via Belmeloro 8/2, I-40126 Bologna, Italy

Contract/grant sponsor: MURST
(*Received 28 July 1997; revised version received 23 February 1998; accepted 29 September 1998*)

 Pestic Sci 0031–613X/99/$17.50

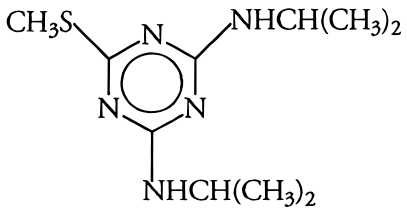

Figure 1. Structure of prometryn (Prom)

phatidylcholine (DPPC) and dipalmitoylphosphatidic acid (DPPA) liposomes for a better evaluation of the mode of action of this herbicide.

2 EXPERIMENTAL

2.1 Preparation of liposomes

Phospholipids (PL) were obtained from Sigma Chemical and prometryn from Riedel de Haen (Pestanal). All chemicals were used without further purification.

Multilamellar liposomes were prepared by mixing chloroform solutions of Prom and PL to reach an appropriate PL/Prom, ratio. The solvent was evaporated under a stream of nitrogen and then under vacuum for 4 h. The DPPC/Prom mixtures were hydrated by adding the buffer solutions. The buffers were: citric acid/sodium citrate (pH 5), sodium dihydrogen phosphate/disodium hydrogen phosphate (pH 7) and boric acid/borax (pH 9). The buffering chemicals were dissolved in saline solution (sodium chlorides 9 g litre^{-1}) and total amount of buffering components was about 10^{-3} M. At these low concentrations, the IR spectra of the buffers did not appear on the IR spectra of DPPC. DPPA and the DPPA/Prom mixtures were hydrated by adding the appropriate amount of sodium hydroxide in saline up to the chosen pH value and then saline solution to give the same volume in all the samples; the total ionic strength of the suspensions was kept constant for each pH value.

2.2 Differential scanning calorimetry

Differential scanning calorimetry (DSC) measures the changes in heat capacity of a system with change in temperature. Any change in state in the system is indicated by a sharp peak whose characteristics give an insight to the nature of the change. DSC has been widely used to examine phase changes occurring in lipids,[8] and is a valuable tool for studying their interaction with other molecules. The parameters usually measured are T_m, the temperature of the transition, $W_{1/2}$, the width of the peak at half-height, and ΔH, the enthalpy change during the transition, determined from the area under the peak.

The lipid/water suspensions were heated in a water bath at about 10°C above the phospholipid transition temperature for 2 h.

Exactly weighed amounts (10 mg) of each lipid suspension were encapsulated in flat-bottomed aluminium pans of 40 µl with crimped on lids.

Differential scanning calorimetric measurements were made in air with a Mettler DSC 20 calorimeter in the 25–95°C temperature range. Heating runs were performed at 2°C min^{-1}. Before each heating run the samples were allowed to stand at 25°C for 30 min, and heating runs were repeated three times to ensure reproducibility of the thermal parameters of the main transition. The quantity of PL used for each calorimetric measurement has been determined colorimetrically as reported elsewhere[7] and in the literature cited therein.

ΔH of fusion was determined by integration of the areas under the melting DSC endotherms following calibration with indium. The errors in enthalpy were less than 5%.

Caprylic acid was used to calibrate the temperature in the range studied.

2.3 Spectroscopic measurements

Vibrational Raman spectra were recorded with a Bruker IFS 66 spectrometer equipped with a FRA-106 Raman module and a Ge diode detector. The excitation source was a Nd^{3+}-YAG laser (1064 nm) in the backscattering (180°) configuration. The focused laser beam diameter was $\approx$100 µm, the spectral resolution 4 cm^{-1}, the encoding interval 1 point cm^{-1} and the apodization function Blackman Harris, 4-term. The laser power was 250 mW on the sample.

Infrared spectra were recorded by a JASCO FT/IR-300E. The spectra were obtained with the ATR technique by using a ZnSe plate (this technique allows aqueous solutions to be studied easily). In order to obtain good quality spectra, 1000 scans for each spectrum were accumulated and computer averaged. The errors in wavenumber were about 0.4 cm^{-1}.

The spectra of the buffers and of water were computer subtracted to obtain the liquid or the lipid-Prom complex spectra.

UV measurements were carried out by means of a JASCO 7850 UV-vis spectrophotometer.

All spectra were measured at room temperature.

3 RESULTS AND DISCUSSION

3.1 Dipalmitoylphosphatidylcholine (DPPC)

DPPC is one of the most common components of biological membranes and can be assumed as a phospholipid model for the uncharged part of a biological membrane. Multilamellar liposomes obtained by DPPC water suspensions are very extensively used as a good model for biological membranes.

The calorimetric data of DPPC and the DPPC–Prom systems (Prom/DPPC molar ratio ranging

Table 1. Calorimetric data of DPPC/Prom system

	pH	T_m(°C)	$W_{1/2}$(°C)	ΔH (kJ mol^{-1})	T_{ptr} (°C)	*Molar ratio* (*Prom*/DPPC)
DPPC	5	41.3	0.7	34.1	36.0	–
DPPC	7	41.4	0.5	34.1	33.9	–
DPPC	9	41.3	0.7	34.2	34.8	–
DPPC + Prom (0.5% wt)	7	41.1	0.5	34.2	32.1	0.015
DPPC + Prom (1% wt)	7	41.0	0.9	34.4	31.6	0.030
DPPC + Prom (2% wt)	7	40.9	1.0	34.2	31.2	0.060
DPPC + Prom (5% wt)	7	40.7	1.1	34.4	31.2	0.150
DPPC + Prom (10% wt)	7	40.0	1.2	34.5	30.5	0.300
DPPC + Prom (20% wt)	5	40.7	1.2	33.2	–	0.600
DPPC + Prom (20% wt)	7	40.0	1.2	34.6	–	0.600
DPPC + Prom (20% wt)	9	39.5	1.2	34.8	–	0.600
DPPC + Prom (40% wt)	7	40.0	1.2	34.6	–	1.200

from 0.015 to 1.2) are given in Table 1. For DPPC the temperature of the main transition (T_m) is 41.4°C and the associated enthalpy change, ΔH, 34.1 kJ mole^{-1}.

In the presence of increasing amounts of Prom, a slight decrease in T_m and a more noticeable lowering in the temperature of the pre-transition (T_{ptr}) were observed; moreover, the pre-transition peak was no longer present when the concentration of Prom reached 20% w/w. These results are in agreement with the idea that, in most cases, the effect of the addition of chemicals to lipid bilayers is more pronounced on the pre-transition than on the main transition.[8]

At pH 7 the main transition peak was fairly symmetrical and its shape did not change as a consequence of the Prom addition, thus indicating that the affinity of Prom toward the hydrophobic moiety of the liposomes in the gel and the liquid crystalline phase is the same.[8] Moreover, the addition of Prom (20% w/w) to neat DPPC caused an increase in the main transition half-width ($W_{1/2}$) from 0.5°C to 1.2°C. Because of the approximately inverse relationship between the number of PL molecules per cooperative unit and $W_{1/2}$,[9] the observed broadening indicates a noticeable lowering of the transition cooperativity of DPPC due to its interaction with Prom.

Changes of pH in the pure DPPC water suspension did not change either the transition temperature or the profile of the endothermic transition, as would be expected for uncharged molecules of DPPC in the 5–9 pH range. On the other hand, pH changes in the same range affected the DPPC–Prom interaction (Table 1); indeed, the temperature of the main endothermic transition increased as the pH was lowered to acid values.

Since the pKa value for Prom is 4.1[10] at pH 5, only a small portion of Prom molecules should be protonated (about 1/10). This is also supported by the infrared spectra of Prom, in which the absorption bands due to the protonated form do not appear, and the spectrum at pH 5 is almost the same as at pH 7 and 9.

Consequently, the DPPC–Prom interaction should not depend on pH in the range under consideration. However, the calorimetric results indicate that the behaviour of Prom at pH 5 is quite different from that at pH 7 and 9.

The thermograms observed after the addition of Prom at pH 9 were slightly skewed towards higher temperatures, suggesting that Prom is distributed preferentially into the gel regions in the membrane.[8]

The lowering of the phase transition temperature (with an increase in pH) without changes in the transition enthalpies indicates that there is an interaction of the herbicide with the polar head group region of DPPC and that a partial penetration of Prom into the hydrocarbon region of the liposomes took place.

In addition, inspection of the calorimetric data reveals that, since an amount of Prom greater than 20% w/w does not influence the PL properties, some saturation effect has taken place.

These findings were confirmed by Raman spectroscopy. This spectroscopic technique gives two order parameters related to the trans/gauche ratio of the acyl chains (C–C stretching region) and the lateral packing of the acyl chains (C–H stretching region).[7] The trans/gauche ratio both in the gel and liquid-crystalline phase did not change as a consequence of Prom addition (20% w/w); the lateral packing decreased by about 5% (from 0.37 to 0.35) in the gel phase and remained constant in the liquid-crystalline phase. These data also confirm that the interaction between DPPC and Prom is limited to the polar moiety of the bilayer.

Vibrational spectroscopy gives further, more detailed insight into the type of interaction between the lipid and the herbicide. The IR and Raman spectra of DPPC and DPPC–Prom water dispersion (20% w/w) at pH 7 are shown in Figs 2(A) and 3(A). Since the DPPC molecules have two carbonyl groups, a doublet should be observed in the carbonyl stretching region; however it has been widely demonstrated that conformational effects are negligible and the presence of more than one band is primarily due to hydrogen bonding and the polarity in

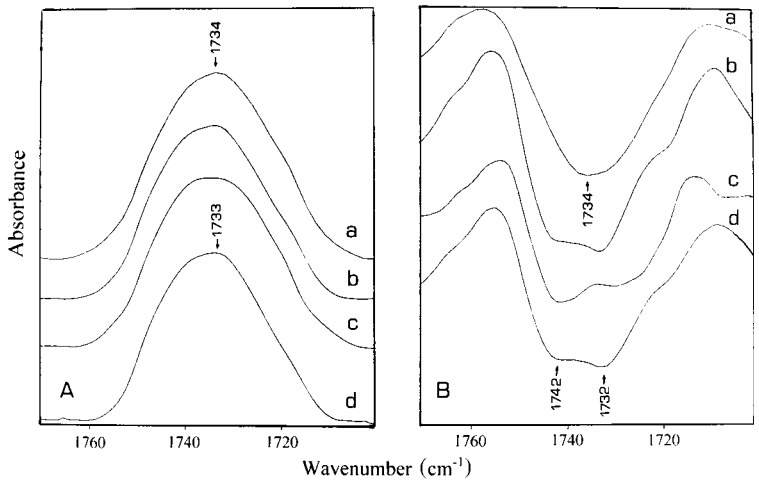

Figure 2. (A) Infrared spectra of the C=O stretching modes of (a) pure DPPC and DPPC Prom (20% wt) water suspensions at (b) pH 9, (c) pH 7 and (d) pH 5, and (B) their second derivative spectra.

the immediate vicinity of the carbonyl groups.[11–13] Only one very broad and fairly symmetric band appeared in both the IR and Raman spectra of neat DPPC at 1734 cm^{-1} and 1738 cm^{-1}, respectively. Furthermore, the second derivative spectra showed only one main component (Figs 2B(a) and 3B(a)). The two carbonyl groups should be equally accessible to water and their chemical environments are equivalent. On addition of Prom (20% w/w), the shape of the vC=O stretching band became asymmetric; the maximum wave number of the IR band remained constant and the Raman wave number decreased to 1735 cm^{-1}. This behaviour can be explained by considering that the asymmetric band is an overlap of two main components whose intensities change as a consequence of the interaction with Prom. These two main components can be distinguished by means of the second derivative spectra (Figs 2(B) and 3(B)). The presence of only one main component in the IR and Raman spectra of neat DPPC and of two main components in the DPPC–Prom spectra indicates that the environments of the two C=O groups of neat DPPC are approximately equivalent, but in the presence of Prom this is no longer the case. This indicates that Prom does not penetrate deeply into the bilayer and is able to modify only the chemical environment of the sn-2 carbonyl group closer to the bilayer interface.[14]

In the N–H stretching region of the IR and Raman spectra of Prom (pH 7) two bands appeared at 3234 and 3091 cm^{-1} (Figs 4(a) and 5(a)). These spectral features are not affected by pH changes; indeed, the same spectra are obtained at pH 5 and pH 9. This splitting cannot be attributed to the presence of two N–H groups. Indeed, as a consequence of deuteration, only the first component was shifted to lower wavenumbers: the spectrum of the deuterated Prom exhibited a band at 2373 cm^{-1}, which agrees well with the calculated value of 2362 cm^{-1}. The low wave number component can be attributed to anharmonic resonances. Possible candidates for the summation tones participating mainly in the low frequency component of the doublet are $1506 + 1588 = 3094\,cm^{-1}$, which are present at about the same wavenumbers in the spectrum of the deuterated Prom.[15]

The relatively low value of the N–H stretching wavenumber indicates that neat Prom is hydrogen bonded as a consequence of the presence of the N–H groups in the molecule. Indeed, vN–H increases to 3440 cm^{-1} in diluted carbon tetrachloride solution, where hydrogen bonds are not present.[15] As a result

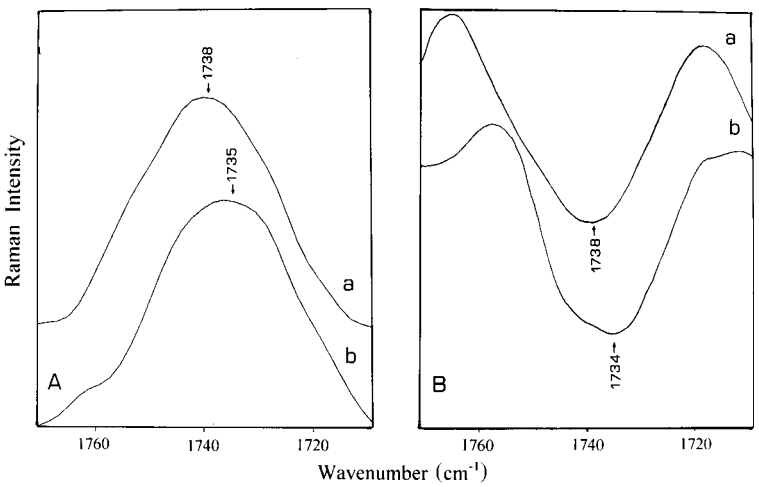

Figure 3. (A) Raman spectra of the C=O stretching modes of (a) pure DPPC and (b) DPPC/Prom (20% wt) water suspensions at pH 7 and (B) their second derivative spectra.

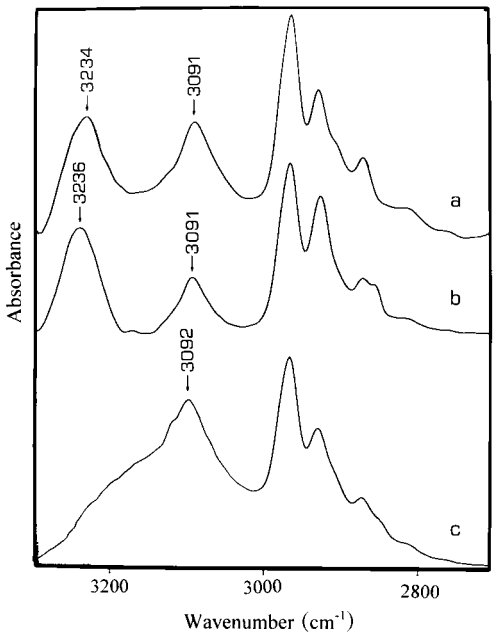

Figure 4. Infrared spectra of the N–H stretching mode of (a) pure Prom in water suspension at pH 7 and Prom in DPPC/Prom (20% wt) water suspensions at (b) pH 7 and (c) pH 5.

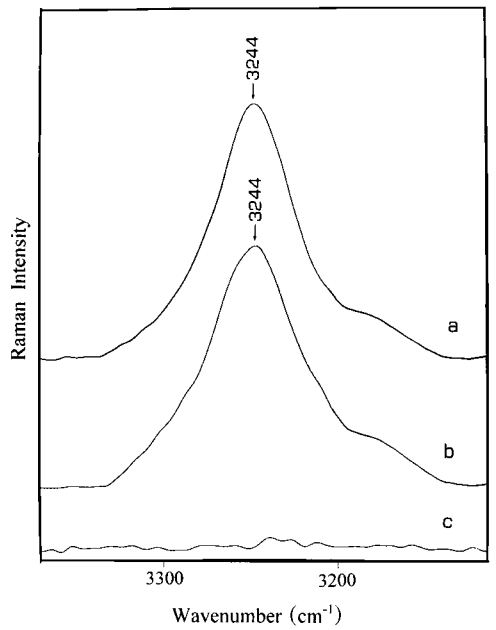

Figure 5. Raman spectra of the N–H stretching mode of (a) pure Prom in a water suspension at pH 7 and Prom in DPPC/Prom (20% wt) water suspensions at (b) pH 7 and (c) pH 5.

of the presence of DPPC at pH 7, the vN–H did not change significantly compared to that of neat Prom (Figs 4(b) and 5(b)). This fact suggest that the strength of hydrogen bonds in neat Prom and in the DPPC–Prom system is about the same.

The wavenumber of vN–H did not change by increasing pH to 9 either in the spectrum of neat Prom or in that of Prom interacting with DPPC.

In the IR and Raman spectra of the DPPC–Prom system at pH 5, the band attributable to vN–H stretching was no longer present (Figs 4(c) and 5(c)); a broad absorption, which modifies the base line of the IR spectrum, was observed around 3000 cm^{-1}. This absorption is typical of protonated amino groups.[16] This fact suggested that the DPPC–Prom interactions change when pH is lowered to acidic values. At these pH values, the protonation of Prom seems to take place. This result is in agreement with the calorimetric data and caused us to suppose that the basicity of Prom increases upon interaction with DPPC.

3.2 Dipalmitoylphosphatidic acid (DPPA)

In resting cells, phosphatidic acids constitute a minor portion of the total phospholipid pool, with moderate biological reactivity. However, being negatively charged at physiological pH, they are very important in interactions with positively charged cations, such as polyamines[17,18] and some herbicides (paraquat, diquat).[7] The functions of phosphatidic acids as intracellular mediators and extracellular messengers of biological activities[19] have also been reported.

At pH 5 and pH 7 the DPPA molecules bear one negative charge, due to the partial ionization of the phosphate group.[20] The complete ionization of the phosphate group occurs at pH 12.[21] Since the total lipid charge depends on pH, the temperature of the melting transition and the associated value of ΔH also depend on pH (Table 2). The transition temperature of DPPA decreases with increase in pH according to the literature data.[20–22] As a consequence of the Prom addition, a slight decrease of T_m was observed: this decrease was 1.7°C at pH 5 and 3.8°C at pH 9. A qualitatively similar behaviour was observed in the DPPC–Prom systems.

The $W_{1/2}$ of the transition remained unchanged suggesting that the addition of Prom is not able to

	pH	T_m(°C)	$W_{1/2}$(°C)	ΔH ($kJ\,mol^{-1}$)
DPPA	5	68.1	3.7	26.4
DPPA	7	64.9	3.6	23.5
DPPA	9	57.5	4.1	21.0
DPPA + Prom (20% wt)	5	66.4	3.6	25.5
DPPA + Prom (20% wt)	7	62.1	3.7	23.7
DPPA + Prom (20% wt)	9	53.7	4.2	22.3

Table 2. Calorimetric data of DPPA/Prom system

change markedly the cooperativity of the transition of DPPA.

The IR and the IR second derivative spectra of DPPA and DPPA–Prom at pH 7 in the vC=O stretching region are illustrated in Fig 6. The band attributable to the vC=O stretching mode of the DPPA ester groups is very broad. A slight shift towards low wavenumbers was observed in the presence of Prom. It seems that Prom affects both carbonyl groups equally, thus indicating that the penetration of Prom into the bilayer can reach both carbonyl groups of DPPA. Indeed, the second derivative spectra showed only one main component in all cases (Fig 6B). This different behaviour could be due both to the expansion of the lipid lattice due to negative charges, which facilitates the insertion of the herbicide into the bilayer and to the different structure of the glycerol backbone in the two lipids.[23]

The spectra of Prom in the vN–H stretching range does not change on the addition of DPPA in the 7–9 pH range; the band attributable to *v*N–H disappears in the presence of DPPA at pH 5.

The vibrational results agree well with those of DPPC and it appears that the two lipids interact with Prom by an analogous mechanism.

4 CONCLUSIONS

The calorimetric and spectroscopic results show that Prom does not perturb the hydrophobic core of the phospholipid (PL) bilayer. As regards DPPC, only a slight decrease of the temperature of the melting process was observed. The ΔH associated with the transition remained constant; the order parameters of acyl chains calculated by means of Raman spectroscopy showed only a very small decrease, which was not particularly significant. This behaviour is in agreement with the results reported by other authors on DPPC-atrazine systems.[24,25]

In the vC=O stretching region of the DPPC spectra, a broad band appeared. Its wavenumber decreased in the Raman spectrum after Prom addition. By means of the second derivative spectra, which allow spectral differences arising from small variations in the structure at the sub-molecular level to be detected and assessed, two main components can be identified. The presence of two main components indicates that the environments of the two C = O groups of DPPC are no longer equivalent; Prom penetrates into the bilayer only until it reaches the more accessible carbonyl group, while the more internal group interacts mainly with the water molecules, as observed in the absence of Prom.

The partition coefficient of Prom (octan-1-ol/water) as deduced by UV measurements depends on the pH of the aqueous solution. At the three considered pH values, comparable results were obtained for pH 7 and 9 (2×10^3). At pH 5 a significantly lower value has been measured (5×10^2); however in this case as well we can suppose that the herbicide could interact with biomembranes. Our results indicate that this interaction is mainly related to the superficial part of the bilayer, with a stronger influence on the C=O ester group nearest to the liposome surface (sn-2).

Calorimetric data indicate that the thermal behaviour of neat DPPC liposomes is not affected by the pH changes; however, a decrease in pH alters the thermal behaviour of the DPPC–Prom systems. At pH 7 and pH 9 Prom is uncharged and is much more effective in lowering the melting temperature of DPPC than at pH 5 when the modified form is present, as suggested by the vibrational spectra in the N–H stretching region.

Since the pKa value of Prom indicates that only a small percentage should be protonated in aqueous solution at pH 5, the spectroscopic behaviour of *v*N–H can be explained only by supposing that the interaction of Prom with the glycerol backbone of PL strongly increases the basicity of the N–H groups.

DPPA, was studied since its negatively charged PL were found to strongly interact with amino groups of polyamines[17,18] and of some herbicides.[7] However, Prom interacts similarly with DPPC and DPPA, and the negative charge on PL seems have a negligible effect.

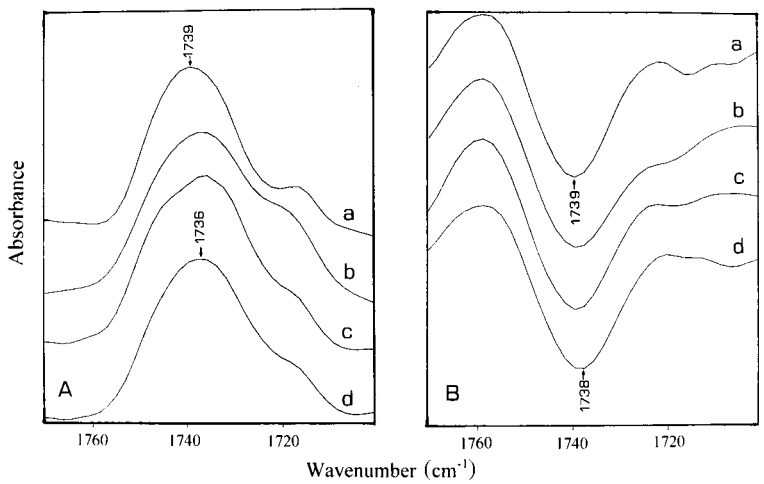

Figure 6. (A) Infrared spectra of the C=O stretching modes of (a) pure DPPA and DPPA/Prom (20% wt) water suspensions at (b) pH 9, (c) pH 7 and (d) pH 5 and (B) their second derivative spectra.

Moreover, it is interesting to note that some herbicides interact mainly with the negatively charged PL;[7] on the other hand, atrazine[24,25] and Prom also interact with uncharged PL. The interaction between Prom and PL is limited mainly to the superficial part of the bilayer. Prom is able to modify the environment of only one ester group in DPPC.

The PL–Prom interaction seems to increase the basicity of the N–H groups of the triazinic herbicide. Analogous effects on the acid-base properties of Prom could take place in the soil by adsorption of the herbicide molecules on clay particles, as has been reported for atrazine.[6]

As a consequence of the changes on lipidic membrane caused by the interaction of Prom with PL, basic mechanisms operating at the membrane level could be affected, for example, the permeability of liposomes to non-electrolytes and the electrolyte diffusion. These interactions could also perturb normal lipid–protein interactions in the modulation of enzyme activity.

ACKNOWLEDGEMENT

This work was supported by grants from MURST 60% and 40% to GF.

REFERENCES

1 Andus LJ, *Herbicides: Physiology*, *Biochemistry*, *Ecology*, Academic Press, London (1976).
2 Erickson JM, Rahire M, Rochaix JD and Mets L, Herbicide resistance and cross-resistance: changes at three distinct sites in the herbicide-binding protein. *Science* (*Washington*) **228**:204–207 (1985).
3 Hirschberg J and McIntosh L, Molecular basis of herbicide resistance in *Amaranthus hybridus*. *Science* (*Washington*) **222**:1346–1349 (1983).
4 Hirschberg J, Bleecker A, Kyle DJ, McIntosh L and Arntzen CJ. The molecular basis of triazine-herbicide resistance in higher-plant chloroplasts. *Z Naturforschung* **39C**:412–420 (1984).
5 Erickson JM, Rahire M, Bennoum P, Delepaire P, Diner B and Rochaix JD, Herbicide resistance in *Chlamydomonas reinhardtii* results from a mutation in the chloroplast gene for the 32-kilodalton protein of photosystem II. *Proc Natl Acad Sci USA* **81**:3617–3621 (1984).
6 Evangelou VP and Wang J, Differences between infrared spectra of atrazine obtained under transmittance and diffuse reflectance modes. *Spectrochim Acta* **49**:291–295 (1993).
7 Bertoluzza A, Bonora S, Fini G, Francioso O and Morelli MA, Interaction of bipyridilium herbicides with model membranes. *Chem Phys Lipids* **75**:137–143 (1995).
8 Biltonen RL and Lichtenberg D, The use of differential scanning calorimetry as a tool to characterize liposome preparations. *Chem Phys Lipids* **64**:129–142 (1993).
9 Mountcastle DB, Biltonen RL and Halsey MJ, Effect of anesthetics and pressure on the thermotropic behavior of multilamellar dipalmitoylphosphatidylcholine liposomes. *Proc Natl Acad Sci USA* **75**:4906–4910 (1978).
10 Tomlin CDS. (ed), *The Pesticide Manual*: *A World Compendium* 11th edn BCPC, Farrham, Survey, UK. pp 1011–1013 (1997).
11 Blume A, Huebner V and Messner G, Fourier Transform Infrared Spectroscopy of $^{13}C=O$ labeled phospholipids hydrogen bonding to carbonyl groups. *Biochemistry* **27**:8239–8249 (1988).
12 Huebner W, Mantsch HH, Paltauf F and Hauser H, Conformation of phosphatidylserine in bilayer as studied by Fourier transform Infrared Spectroscopy. *Biochemistry* **33**:320–326 (1994).
13 Lewis RNAH, McElhaney RN, Pohle W and Mantsch HH, Components of the carbonyl stretching band in the infrared spectra of hydrated 1,2-diacylglycerolipid bilayers: a reevaluation. *Biophys J* **67**:2367–2375 (1994).
14 Hauser H, Pascher I, Pearson RH and Sundell S, Preferred conformation and molecular packing of phosphatidylethanolamine and phosphatidylcholine. *Biochim Biophys Acta* **650**:21–51.
15 Bonora S, Fini G and Torreggiani A, Interaction of herbicide triazine derivatives with model membranes. *Spectroscopy of Biological Molecules*, ed. Merlin JC, Turrells and Huvenne JP, Kluwer Academic Pub. Dordrecht. pp 415–416 (1995).
16 Bellamy LJ, *Advances in Infrared Group Frequencies*, Methuen and Co Ltd, Bungay, Suffolk, UK (1968).
17 Young MW and Green C, The binding of polyamines to phospholipid bilayers *Biochem Pharmacol* **22**:4037–4041 (1986).
18 Bertoluzza A, Bonora S, Fini G and Morelli MA Spectroscopic and calorimetric behaviour of phospholipid–polyamine molecular interaction. *J Raman Spectrosc* **19**:369–373 (1988).
19 English D, Cui Y and Siddiqui RA, Lipids messenger functions of phosphatidic acid. *Chem Phys Lipids* **80**:117–132 (1996).
20 Jacobson K and Papahadjopoulos D, Phase transition and phase separations in phospholipid membranes induced by changes in temperature, pH and concentration of bivalent cations. *Biochemistry* **14**:152–161 (1975).
21 Blume A, Apparent molar heat capacities of phospholipids in aqueous dispersion. Effects of chain length and head group structure. *Biochemistry* **22**:5436–5442 (1983).
22 Van Dijck PWM, De Kruijff B, Verkleij AJ, Van Deenen LLM and DeGier J, Comparative studies on the effects of pH and calcium ions on bilayers of various negatively charged phospholipids and their mixtures with phosphatidylcholine. *Biochim Biophys Acta* **512**:84–96 (1978).
23 Harlos K, Eibel H, Pascher I and Sundell S, Conformation and packing properties of monosodium dimyristoylphosphatidate. *Chem Phys Lipids* **43**:115–126 (1984).
24 Tanfani F, Ambrosini A, Albertini G, Bertoli E, Curatola G and Zolese G, Interaction of the herbicide atrazine with model membranes I: Physicochemical studies on dipalmitoyl phosphatidylcholine liposomes. *Chem Phys Lipids* **55**:179–189 (1990).
25 Zolese G, Ambrosini A, Bertoli E, Curatola G and Tanfani F, Interaction of the herbicide atrazine with model membranes II: Effect of atrazine on function of phospholipid vesicles. *Chem Phys Lipids* **56**:101–108 (1990).